First insights into the operational mode of epithelial peptide transporters

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Peptide transporters in the apical membrane of intestinal and renal epithelial cells are responsible for absorption and reabsorption of di- and tripeptides and peptidomimetics. There are two distinct genes coding for the intestinal (PepT1) and renal (PepT2) isoforms of peptide transporters. Differences in the gene products include structural aspects, molecular mass and glycosylation, as well as functional characteristics (different affinity types). Both transporter isoforms mediate rheogenic transport of peptide substrates by coupling the substrate flux to an inwardly directed proton gradient with the driving force provided mainly by the inside-negative transmembrane electrical potential.

What makes the peptide transporters a novel class of mammalian solute transporters is (a) their use of H^+/H_3O^+ ions rather than Na^+ ions as the cotransported ion species and (b) their capability for binding and translocating stereospecifically an enormous number and variety of substrates (400 dipeptides, 8000 tripeptides). Since around 20% of the natural substrates carry net positive or negative charges at physiological pH, one of the most interesting questions is how the peptide carriers handle neutral and charged substrates.

Three groups have independently addressed this fascinating question using similar techniques in oocytes expressing the rabbit or human PepT1 (Mackenzie *et al.* 1996*a*; Amasheh *et al.* 1997; Steel *et al.* 1997). In the studies reported by Steel *et al.* (1997) in this issue of *The Journal of Physiology* the two-electrode voltage-clamp technique, in conjunction with measurements of pH_i, was used to assess dipeptide-dependent changes in membrane potential, as well as generated inward currents under voltage-clamp conditions and intracellular H^+ activity during transport of neutral (Gly-Leu), cationic (Gly-Lys) and anionic (Gly-Glu) dipeptides. The rabbit PepT1 was shown to transport all substrates, regardless of their net charge in solution, by an electrogenic mechanism with a concomitant reduction in pH_i, suggesting that the peptide-evoked charge fluxes are due to the movement of H^+/H_3O^+. Similar observations were made by other investigators (Mackenzie *et al.* 1996*a*; Amasheh *et al.* 1997). Whereas I_{max} currents in voltage-clamped oocytes were essentially pH_o independent, apparent substrate affinities were affected by extracellular pH and membrane potential (Mackenzie *et al.* 1996*b*; Amasheh *et al.* 1997). Consequently, peptide-evoked currents at low substrate concentrations showed pronounced pH dependence with different pH optima for the zwitterionic and charged substrates (Amasheh *et al.* 1997; Steel *et al.* 1997).

Based on the data obtained by combining flux studies of radiolabelled charged dipeptides with electrophysiology and pH_i measurements, Steel *et al.* (1997) conclude that electrogenicity of transport is achieved by different peptide–H^+ flux coupling ratios. Whereas neutral and cationic dipeptides reveal a 1:1 stoichiometry, a more rapid intracellular acidification in the case of the acidic dipeptide supports the hypothesis of a 1:2 (2 H^+ ions) coupling ratio. There are, however, two possible explanations for the faster initial decline in pH_i derived from investigation of the transport of acidic substrates. Decreasing pH_o not only affects the transporting protein and its substrate affinity but also the degree of dissociation of the substrate. For anionic compounds, lowering pH_o increases the percentage of its zwitterionic form which generally appears to have a higher affinity for the transporter than its charged counterpart. A higher initial acidification rate could therefore, as suggested by the authors, result from influx of the zwitterionic form of Gly-Glu* at low pH_o followed by deprotonation of the substrate by entering the less acidic intracellular environment. This, however, would mean that the acidic substrates are partly or mainly transported in the neutral form with a 1:1 (peptide:H^+) stoichiometry during translocation, but deliver more than one acid equivalent to the intracellular compartment during a cycle. With respect to changes in pH_i induced by peptide transport, one should also consider that the rapid hydrolysis of peptides consisting of L-α-amino acids within cells can lead to an acidification that may contribute significantly to the overall rate of intracellular acidification during transport.

It should be emphasized here that the proposed 1:1 charge:substrate flux coupling ratio which is independent of the substrate charge has also been observed in two other recent studies (Mackenzie *et al.* 1996*a*; Amasheh *et al.* 1997). Therefore, the function of PepT1 seems to be determined mainly by its charge transfer capacity and the adaptational changes in proton and substrate binding characteristics (Fig. 1). The significance and value of the study by Steel *et al.* (1997) is that for the first time different techniques have been used to gain insights into the handling and operational mode of a novel solute transporter that is capable of translocating a variety of differently charged substrates in an electrogenic transport step. Together with the findings of other recent studies on PepT1 function, a first picture is emerging according to which a membrane potential-dependent H^+ binding process represents the initial step. It is followed by potential-dependent substrate binding and formation of a ternary substrate–carrier complex that undergoes a conformational change and delivers a substrate molecule, a water molecule and a net positive charge across the plasma membrane.

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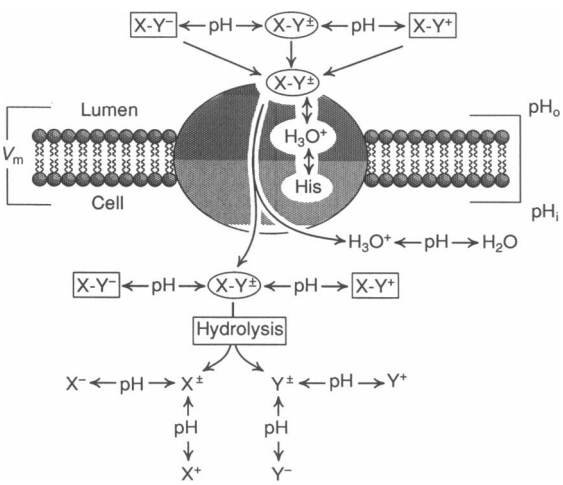

Figure 1. Diagram representing the suggested operational mode of the intestinal peptide transporter

After membrane potential-dependent binding of H^+/H_3O^+ to PepT1 the peptide (with a preference for the zwitterionic form) is bound depending on membrane potential. Then both peptide and H^+/H_3O^+ are delivered to the internal face. The confomational change of the ternary complex causes membrane depolarization regardless of the substrates initial charge. Intracellular acidification is a consequence of H^+/H_3O^+ influx but may additionally be increased by hydrolysis of the transported peptides. Whereas the membrane potential determines the maximal transport velocity, extracellular and intracellular pH affect both substrate binding and release as well as the rate of hydrolysis. His residues in the active site of PepT1 are proposed to affect substrate binding and charge translocation (Terada *et al.* 1996; Steel *et al.* 1997). X-Y± encircled represents the zwitterionic dipeptide consisting of two different L-α-amino acids; X± and Y± are the corresponding free amino acids.